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| 13. SUPPLEMENTARY NOTES | | | | | |
| 14. ABSTRACT: The goal of this proposal is to determine whether hydrophilic carbon clusters (HCCs), that have exhibited potent antioxidant properties, can promote motoneuron integrity and enhance survival in a mouse model of amyotrophic lateral sclerosis. This project involves work performed at both UT-Health and Rice University; combining the cutting edge nanotechnology expertise of Dr. James Tour's laboratory at Rice with Dr. Grill's animal models at UT-Health. We are attempting to, first, determine whether Dr. Tour's HCCs can enhance motoneuron survival as well as overall lifespan in the G93A mouse model of ALS. In addition, we will also determine whether the HCCs, when combined with the current "gold standard" treatment for ALS, riluzole, will represent a new combinatorial therapy to improve outcome for patients living with ALS. We will describe the current state of the project as well as describe a significant setback encountered in year 1 (see below). | | | | | |
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INTRODUCTION

The goal of this project is to develop a novel therapy that will maintain functional behavior and enhance lifespan for those living with the disease, amyotrophic lateral sclerosis (ALS). Currently, riluzole is the only FDA-approved therapeutic for ALS but provides only a few months enhancement in lifespan. In collaboration with the laboratory of Dr. James Tour at Rice University in Houston, we are exploring the use of a novel nanovector as a putative treatment for ALS. Hydrophilic carbon clusters (HCCs) are single-walled carbon nanotubes that have been shown to possess potent anti-oxidant properties both in vitro and in vivo. Our goal is to determine whether HCCs can improve morphological, functional and overall survival when used in the G93A mouse model of ALS. HCCs are delivered to mice only after they begin to exhibit signs of ALS disease progression (to more accurately mimic human clinical conditions). HCCs are delivered via osmotic minipumps to the jugular vein in order to attain a constant application of treatment. We will determine whether HCC treatment can preserve motor function as well as motor neuron survival. We are also assessing whether HCCs can enhance overall survival of the G93A mouse compared to pump-delivered vehicle. Subsequent to these experiments, we will also determine whether a combination of HCCs with systemically-delivered riluzole can enhance the survival-promoting properties of riluzole. If successful, we will have succeeded in developing a novel combination therapy that will enhance the treatment efficacy of the only currently FDA-approved treatment for ALS.

BODY:

The overall hypothesis of this project is that anti-oxidant nanovectors can be targeted to enhance delivery of Riluzole into the CNS in a mouse model of ALS. In addition, the antioxidant properties of the nanovectors will preserve locomotor behavior and motoneuronal survival in this model. Finally, the dual antioxidant/Riluzole therapy will produce a greater improvement of both lower motoneuron survival and functional improvement than either intervention applied individually.

This hypothesis is currently being tested through two specific aims:

Specific Aim 1: Determine whether antioxidant PEG-HCCs functionalized with an antibody against the transferring receptor can improve behavioral outcome and enhance overall survival when delivered through a sustained, intravenous route using the G93A-SOD1 mutant mouse model of ALS. Over a 6 week period, mutant mice with ALS-like symptoms will receive intravenously either PEG-HCCs, antibody functionalized PEG-HCCs or vehicle via jugular-catheterized osmotic minipump. Outcome measures will include assessment of locomotor activity via PAS activity boxes, the ALS Neurological Score and longitudinal changes in body weight. Cohorts will be sacrificed at days 100 and 120 for the purpose of performing assessments of lower motoneuron survival in the ventral horns of the lumbar spinal cord.

Specific Aim 2: Determine whether the systemic delivery of Riluzole into the CNS can be improved by antibody-functionalized PEG-HCCs, resulting in improved behavioral outcome and enhanced overall survival when delivered intravenously to the G93A-SOD1 mutant mouse model of ALS. As above, mutant mice with ALS-like symptoms will receive intravenously either 1) Riluzole at 10 mg/kg, 2) PEG-HCCs, 3) an antibody against the transferrin receptor, 4) Riluzole at 10 mg/kg mixed with an antibody against the transferrin receptor, 5) PEG-HCCs functionalized with an antibody against the transferrin receptor, 6) Riluzole loaded onto PEG-HCCs, 7) Riluzole loaded onto PEG-HCCs functionalized with an antibody against the transferrin receptor and 8) vehicle. Behavioral outcome measures will include will be the same as described in Aim 1. Cohorts will be sacrificed at days 100 and 120 to measure the amount of Riluzole in lumbar spinal cord via High Performance Liquid Chromatography.

Progress: Due to repeated problems encountered with Jackson Laboratories, our source for the establishment and maintenance of the G93A ALS mouse model, we experienced a significant setback in year 1 that has resulted in a loss of over 6 months time. On two separate occasions during year 1, Jackson Laboratories provided us with the incorrect animal subjects that we required for our breeding colony. This resulted in a lack of useable research subjects. We have attempted repeatedly to receive compensation from Jackson Laboratories for lost time and resources, but have been unsuccessful. This has only become more frustrating when this issue was repeated. We experienced significant lost time not only in terms of the necessary breeding, but in the time that it took to get Jackson Laboratories to admit to the problem and provide us with replacement animals. While the replacements have certainly helped us get back on track, they were unwilling to provide compensation for the lost effort as well as other financial costs (animal per diem charges, for instance). Currently, we have dramatically

expanded our colony in order to achieve our original goals in the time remaining for this project. Currently, we are focused on Aim 1; specifically determining whether HCCs can enhance overall survival. We have chosen this outcome to initially focus upon as we believe that success in this sub aim will hold the greatest clinical relevance. This survival study (HCCs vs vehicle) currently has numbers of 20 mice per group (again, HCCs vs vehicle) and should be completed by the end of November. In addition to survival, locomotor behavior is also being assessed as a function of time in this study. While this study approaches completion, additional cohorts of subjects have been generated for the purpose of assessing motoneuron survival at day 110. These animals should be grafted in December.

Subsequent to the studies that focus only on HCCs vs vehicle, we anticipate having sufficient time to perform the survival comparisons of HCCs-alone vs riluzole vs HCCs + riluzole as well as the proposed motoneuron survival and riluzole bioavailability studies prior to the end of July 2014. The Tour laboratory has provided us with HCCs as requested.

Key Research Accomplishments:

Due to the issues described above involving Jackson Laboratories, our main accomplishment involves rebuilding the animal colony and successfully carrying out the proposed comparison of HCCs vs. vehicle survival study. Our goal will be to have a clear result to report on HCC-mediated survival by the next progress report period.

Reportable Outcomes:

Based on the model development underlying this proposal, the PI of this proposal has submitted one other application to the DOD's ALSRP mechanism entitled "Targeting AMPA and NMDA glutamate receptors using cutting edge aptamers technology to suppress excitotoxicity and improve outcomes in G93A mouse model of ALS". In addition, Dr. Grill is serving as Co-PI on a new DOD-ALS proposal from a junior investigator, Qing Yang, M.D., from the Department of Integrative Biology and Pharmacology at UT-Health. The goal of this application is to assess the potential efficacy of a novel NMDA receptor subunit inhibitor in treating ALS. Both grants are currently pending with the CDMRP.

Conclusion:

Based on the issues described above, it is premature to formulate any conclusions based on the potential efficacy of HCCs as a therapeutic for ALS. We have, however, dramatically increased the size of our colony of ALS mice for the purpose of achieving our overall goals during this final year of the project.